REMARKS

Claims 20, 24, 26 and 33-38 are pending in the subject application. Claims 24, 26 and 33-38 are allowed. By this amendment, applicants have amended Claim 20. The amendment places the application in condition for allowance or in better form for appeal. Applicants maintain that the amendment to Claim 20 does not raise an issue of new matter. Support for the amendment to Claim 20 can be found at least in the specification on page 2, lines 30-34, and in the previous version of the claim. Accordingly, entry of the amendment is respectfully requested.

Rejection of Claim 20 under 35 U.S.C. §103(a)

Claim 20 is rejected under 35 U.S.C. §103(a) as unpatentable over Olsson et al. (J. Appl. Biochem. 5:437-445, 1983) ("Olsson"), in view of Cruse et al. (Illustrated Dictionary of Immunology, 1995) ("Cruse").

Applicants respectfully traverse this rejection and maintain that Claim 20 is patentable over the cited references.

Olsson measured the accumulation of total adenylate kinase and hemoglobin in the plasma of preparations of red blood cells stored for as long as 41 days. Olsson described that there was a high degree of correlation between the amount of accumulated hemoglobin and adenylate kinase in the plasma of the stored units of red blood cells. Olsson does not teach or suggest that erythrocyte adenylate kinase can be used as a marker for diagnosing erythrocyte hemolysis *in vivo*.

In the current Office Action, the Examiner noted that rejected Claim 20 does not recite the feature that erythrocyte hemolysis occurs *in vivo*. In reply, applicants have hereinabove amended Claim 20 to be directed to a method for diagnosing erythrocyte

hemolysis in a subject who is suspected of having erythrocyte hemolysis *in vivo*, the method comprising the steps of:

(a) obtaining a serum sample from a subject who is suspected of having erythrocyte hemolysis *in vivo*; and

(b) determining the level of erythrocyte adenylate kinase activity in said sample, the presence of at least about 20 U/L erythrocyte adenylate kinase activity in said sample being indicative of erythrocyte hemolysis *in vivo* in said subject.

This diagnosis is important to enable a physician to determine an appropriate treatment regime for the subject, as discussed for example on page 1 of the application. This is a different problem from the application that motivated Olsson's study, which evaluated the viability of stored erythrocytes due to concerns about the possible toxic effects due to the release of intracellular contents in blood stored in blood banks for use in blood transfusion (Olsson, p. 437, first paragraph).

The existence of markers for hemolysis of erythrocytes in a blood storage bag, as taught by Olsson, is not necessarily predictive of the usefulness of the same markers for diagnosing erythrocyte hemolysis in a subject who is suspected of having erythrocyte hemolysis *in vivo*. The natural clearance of blood proteins *in vivo* does not take place in a blood storage bag. In addition, not all red blood cells proteins are effective diagnostic markers for hemolysis *in vivo*.

As discussed previously, red blood cells contain a number of proteins including hemoglobin, acetylcholinesterase, adenylate kinase, aldolase, aspartate aminotransferase, creatine kinase, glucose-6-phosphate dehydrogenase, hexokinase, lactate dehydrogenase, malate dehydrogenase, phosphohexose isomerase, and pyruvate kinase (e.g., see Lindena et al., J. Clin. Chem. Clin Biochem. 24: 49-59 and 61-71, 1986,

of record). The skilled artisan might consider any of the proteins contained within red blood cells as potential markers for hemolysis *in vivo* since a rupture of the red blood cells might release many of the proteins contained within the red blood cells. Nevertheless, not all red blood cell proteins are diagnostic for hemolysis *in vivo*. As discussed more fully in applicants' October 14, 2004 reply, the body's metabolism of red blood proteins is complex. By way of example, hemoglobin resulting from low levels of hemolysis is thought to form a complex with haptoglobin in plasma, which is subsequently removed by hepatic parenchymal cells. As such, hemoglobin cannot serve as a marker for low levels of hemolysis *in vivo*. In addition, plasma hemoglobin levels are normal in patients with most hereditary hemolytic anemias (Crosby et al. J Lab Clin Med 38:829-41: 1951, of record). Serum levels of lactate dehydrogenase, an intracellular component of red blood cells, are elevated in hemolysis, but may also be elevated in hepatic, cardiac, pulmonary, and placental diseases, thus reducing the test's specificity for detecting hemolysis. In addition, lactate dehydrogenase levels are inconsistently variable in conditions of extravascular hemolysis.

In the current Office Action, the Examiner indicated that the obviousness rejection is also based on the teaching of Olsson that adenylate kinase activity is suitable for monitoring lysis of cells regardless of the presence of hemoglobin, as in the case of non-erythrocytes. In this regard, applicants note that Olsson states that "The [adenylate kinase] activity is extremely stable to storage ..., which makes adenlyate kinase suitable for monitoring cell lysis... The lytic processes of cells not containing hemoglobin could probably also be studied." (See page 437, 2nd full paragraph, emphasis added.) Furthermore, Olsson indicates that "Further work is underway to use this assay for studies of lytic processes during storage of platelets and granulocytes." (See page 445,

last sentence, emphasis added.) Thus, Olsson teaches that work is underway to use an adenylate kinase assay to study lysis of non-erythocytes during storage conditions. These statements by Olsson do not teach or suggest that erythrocyte adenylate kinase can be used as a marker for diagnosing erythrocyte hemolysis *in vivo* for reasons discussed above.

Applicants note that Cruse et al. was cited by the Examiner only for the purpose of teaching a serum sample as an alternative to a plasma sample.

In view of the remarks and amendments made hereinabove, applicants submit that the invention set forth in Claim 20 is patentable over the cited references. Accordingly, reconsideration and withdrawal of this ground of rejection are respectfully requested.

CONCLUSIONS

In view of the amendments and remarks made herein above, reconsideration and withdrawal of the rejection in the April 19, 2005 Final Office Action and passage of all of the pending claims to allowance are respectfully requested. If there are any minor matters that prevent allowance of the subject application, the Examiner is requested to telephone the attorneys listed below.

No fee is deemed necessary in connection with the submission of this response. However, if any fee is required to maintain the pendency of the subject application, the Patent Office is authorized to withdraw the amount of any such fee from Deposit Account No. 01-1785.

Respectfully submitted,

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